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Nitrosyl Transfer to Metalloproteins. Selective Intermolecular Transfer of the Nitrosyl Group from Cobalt Nitrosyls to Hemoglobin and Myoglobin

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Nitrosyl complexes of numerous metalloproteins, particularly the iron hemoproteins, have been prepared and are well characterized [1–3]. Direct combination of nitric oxide with metalloproteins is universally employed to prepare these complexes. Recently, intermolecular transfer of the nitrosyl ligand from a limited number of metal nitrosyls to a variety of transition metal complexes (eqn. 1) has been reported [4–6].

$$M(NO) + M' \longrightarrow M + M'(NO) \quad (1)$$

Although few examples currently exist for nitrosyl transfer to iron(II) and to iron(III) systems [7], the rapid rates that are characteristic of reactions of hemoproteins with nitric oxide [8, 9] and the stabilities of hemoprotein iron nitrosyl complexes suggests the feasibility of nitrosyl transfer to iron hemoproteins if the nitrosyl transfer reagent can obtain access to the heme iron.

Experimental

Hemoglobin (type IV), myoglobin (type III), horseradish peroxidase (type II) and cytochrome c (type II-A) were obtained from Sigma Chemical Co. and were further purified by previously reported procedures [1–3]. Standard procedures were employed to prepare aqueous solutions of the purified metalloproteins in deoxygenated phosphate buffer (c, 0.05 *M; p*H, 7.0), and the nitrosyl complexes of each of the hemoproteins were prepared by direct combination with nitric oxide [10]. Their characteristic ultraviolet and visible spectra were identical to those previously reported [1–3, 11].

Nitrosyl transfer reagents $Co(NO)D_2 \cdot MeOH$ (D = dimethylglyoximate) [4], $Co(NO)T_2 \cdot MeOH$ (T = tetramethylenegloyoximate) [12], $[CoCl(NO)(en)_2]ClO_4$ (en = ethylenediamine) [13], and the black $[Co(NH_3)_5NO]Cl_2$ [14] were prepared by standard procedures. Spectroscopic analyses were employed for structural identification, and elemental analyses provided additional structural confirmation for $Co(NO)T_2 \cdot MeOH$. The formation of hemoprotein nitrosyl complexes from reactions with these cobalt nitrosyls was determined by ultraviolet and visible spectral analyses through spectral comparisons with the hemoprotein nitrosyls produced by direct combination with nitric oxide. With the exception of black $[Co(NH_3)_5No]Cl_2$, these cobalt nitrosyl complexes showed no tendency to dissociate nitric oxide under the reaction conditions employed in this study.

Rates for nitrosyl transfer were determined at 10.0 °C by monitoring the decrease in absorbance of the Soret band for deoxyhemoglobin at 430 nm with time. A minimum of five kinetic runs was used to determine the rate of nitrosyl transfer from the cobalt nitrosyls to deoxyhemoglobin. Initial deoxyhemoglobin concentrations were within the range $(0.7–1.8) \times 10^{-5}$ *M*; initial cobalt nitrosyl concentrations were between 5- and 10-times those of deoxyhemoglobin. The precision of analysis was ±4% of the reported values at maximum deviation. Kinetic rate constants were reproducible through at least two different preparations of hemoglobin and of the cobalt nitrosyl reagent.

Results and Discussion

Hemoglobin A, methemoglobin, metmyoglobin, and the iron(II) and iron(III) horseradish peroxidase and cytochrome c hemoproteins were chosen as potential nitrosyl acceptors because of their desparate specific functions. Treatment of this selection of hemoproteins at 25 °C under rigorously deoxygenated conditions with stoichiometrically equivalent amounts (based on heme iron) of the nitrosyl transfer reagents $Co(NO)D_2$, $[CoCl(NO)(en)_2]ClO_4$, and the black $[Co(NH_3)_5NO]Cl_2$ produced the striking pattern of results that is summarized in Table I. Complete stoichiometric nitrosyl transfer occurred with those hemoproteins that were observed to form their corresponding nitrosyl complexes upon treatment with the nitrosylcobalt reagents. The absence of nitrosyl transfer by $Co(NO)D_2$ and $[CoCl(NO)(en)_2]ClO_4$ to hemoproteins other than hemoglobin and myoglobin was not due to the occurrence of competing reactions since these cobalt reagents could be observed to undergo nitrosyl transfer to hemoglobin following combination with the unreactive hemoprotein in solution. The use of a 10-fold molar excess of either $Co(NO)D_2$ or $[CoCl(NO)(en)_2]$ did not effect even partial nitrosyl transfer to those hemoproteins that were unaffected by stoichiometric amounts of these reagents. The cobalt product from nitrosyl transfer according to nitrosyl/ligand interchange (*eg.*, $CoD_2 \cdot 2H_2O$), although not separable

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TABLE I. Comparison of Nitrosyl Hemoprotein Formation with Cobalt Nitrosyls and Nitric Oxide.

Hemoprotein[a]	Product from Combination with		
	$Co(NO)D_2$	$[CoCl(NO)(en)_2]ClO_4$	$[Co(NH_3)_5NO]Cl_2$[b]
Hb	HbNO	HbNO	HbNO
MHb	MHb	MHb	MHbNO
Mb	MbNO	MbNO	MbNO
MMb	MMb	MMb	MMbNO
$HRP(Fe^{II})$	$HRP(Fe^{II})$	$HRP(Fe^{II})$	$HRP(Fe^{II})NO$
$HRP(Fe^{III})$	$HRP(Fe^{III})$	$HRP(Fe^{III})$	$HRP(Fe^{III})NO$
Cyt $c(Fe^{II})$[c]	Cyt $c(Fe^{II})$	Cyt $c(Fe^{II})$	Cyt $c(Fe^{II})$
Cyt $c(Fe^{III})$	Cyt $c(Fe^{III})$	Cyt $c(Fe^{III})$	Cyt $c(Fe^{III})NO$

[a]Abbreviations: Hb = hemoglobin A, MHb = methemoglobin, Mb = myoglobin, MMb = metmyoglobin, HRP = horseradish peroxidase, Cyt c = cytochrome c. [b]Identical results were obtained in reactions with gaseous nitric oxide. [c]Reaction performed at *p*H 7.0.

from hemoglobin by chromatographic means, was shown in separate experiments to produce no observable change in the electronic spectrum of hemoglobin.

Nitrosyl transfer from $Co(NO)D_2$, $Co(NO)T_2$, and $[CoCl(NO)(en)_2]ClO_4$ to hemoglobin A is characterized by well-defined second-order kinetics, first order in the cobalt nitrosyl complex and first order in hemoglobin. Quantitative formation of nitrosylhemoglobin was observed in each of these transformations. The observed second order rate constants for nitrosyl transfer to hemoglobin at 10.0 °C are $2.05 \times 10^2\ M^{-1}\ s^{-1}$ ($CO(NO)D_2$), $0.86 \times 10^2\ M^{-1}\ s^{-1}$ ($Co(NO)T_2$), and $0.72 \times 10^2\ M^{-1}\ s^{-1}$ ($[CoCl(NO)(en)_2]ClO_4$).

Two limiting mechanisms for nitrosyl transfer are evaluated in this study. The liberation of nitric oxide from the metal nitrosyl prior to nitric oxide association with the metalloprotein provides one possible pathway for nitrosyl transfer. In such a case, the liberation of nitric oxide may be rate-limiting, particularly in view of the exceedingly rapid rate for nitric oxide association with hemoglobin ($k = 25 \times 10^6\ M^{-1}\ s^{-1}$ at 20 °C) [8]; however, relative to the direct use of nitric oxide, nitrosyl transfer from a metal nitrosyl would not exhibit any selectivity in reactions with metalloproteins. In an alternative mechanism, direct transfer of the coordinated nitrosyl group from the metal nitrosyl to the metalloprotein occurs without the intervention of unassociated nitric oxide. Selective nitrosyl transfer and kinetic dependence on both the metal nitrosyl and the metalloprotein are predictable characteristics of this pathway.

The kinetic results and specificity of nitrosyl transfer from $Co(NO)D_2$, $Co(NO)T_2$, and $[CoCl(NO)(en)_2]ClO_4$ demonstrate that these metal nitrosyls effect direct intermolecular transfer of the nitrosyl ligand to hemoglobin. These nitrosyl complexes are stable to nitric oxide dissociation in the buffered aqueous media, and they are unaffected by metalloproteins other than those to which these complexes transfer the nitrosyl ligand. The dissimilarity of the second order rate constants for nitrosyl transfer from these cobalt nitrosyls to hemoglobin indicates that access to the heme iron is selectively impeded by steric and electronic differences in the cobalt nitrosyl complexes. Indeed, between the electronically similar $Co(NO)D_2$ and $Co(NO)T_2$, the sterically more voluminous $Co(NO)T_2$ undergoes nitrosyl transfer 2.4-times slower than does $Co(NO)D_2$.

The black $[Co(NH_3)_5NO]Cl_2$ has been reported to serve as an effective nitrosylating reagent for iron(II) [5]. Although generally included in the presently limited classification of reagents that undergo intermolecular nitrosyl transfer [6, 7], the mechanism of nitrosyl transfer by this reagent has not been definitively established because of the extraordinary susceptibility of black $[Co(NH_3)_5NO]Cl_2$ to decomposition in water [15]. However, comparison of the results obtained for nitrosyl transfer from the analogous $[CoCl(NO)(en)_2]ClO_4$, from the black $[Co(NH_3)_5NO]Cl_2$, and by direct combination of nitric oxide with the selection of hemoproteins listed in Table I provides reasonable assurance that the black pentaamine nitrosyl complex undergoes initial decomposition with water to liberate nitric oxide which rapidly diffuses to the heme iron rather than direct intermolecular nitrosyl transfer from cobalt to the hemoprotein. Since decomposition of the black $[Co(NH_3)_5NO]Cl_2$ occurs with stoichiometric liberation of nitric oxide, this stable solid nitrosyl complex can be employed in a convenient and quantitative procedure for the preparation of metal nitrosyls. Even iron(III) hemoproteins such as methemoglobin are conveniently nitrosylated by addition of the aqueous metalloprotein solution to a stoichiometric amount of the solid nitrosyl complex.

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